Winemaking waste as antimicrobial against the foodborne pathogen *Campylobacter*.

A. J. Martínez-Rodríguez[1], E. Mingo[1], J.M. Silván[2], S. de Pascual Teresa[2], A. V. Carrascosa[1].

1- Institute of Food Science Research (CIAL), Spanish Council for Scientific Research (CSIC) - Universidad Autónoma de Madrid (UAM).
2- Institute of Food Science, Technology and Nutrition (ICTAN), Spanish Council for Scientific Research (CSIC).

Introduction

Campylobacter is considered to be the most important causal pathogen of food-borne gastrointestinal illnesses worldwide, with poultry, and especially chicken, being the main source of infection in humans (1). Since a large proportion of the European Union chicken production is contaminated with the pathogen (2), it is essential to search for new, natural and sustainable strategies to reduce the incidence of this bacteria in the food chain. Winemaking waste (WW) is a by-product of winemaking formed by grape pomace (the mashed up skins, seeds, and stems) and residual yeast. It contains high amounts of phenolic compounds which are a valuable source of bioactive compounds. In the present work, different extracts were obtained from WW (Tempranillo grape) to evaluate their antimicrobial effect on *Campylobacter*

Objectives

The objectives of the present study were: (a) to evaluate the antimicrobial activity of WW against *Campylobacter spp,* (b) to determine the main phenolic composition of the extract, and c) to confirm the behavior observed using phenol pure standards.

Materials & Methods

Extract preparation & Evaluation of the Antimicrobial activity of WW

- 100 gr of homogenized Tempranillo WW
- Methanolic and aqueous extraction of the phenolic compounds
- Total phenolic amount estimated by Folin assay

- Seven strains of *C. jejuni* and one strain *of C. coli*
- Exposure to WW at different concetrations in Brucella Broth (BB). Incubation in VAIN at 42ºC under microaerophilic conditions (85% N_2, 10% CO_2, 5% O_2).
- Recovery media: Müeller Hinton Agar (MHA)

Structural analysis of the phenolic composition of the extracts

- The main phenolic compounds present in the WW were fractioned by semi-preparative reverse-phase high-performance liquid chromatography (RP-HPLC)
- Main phenolic compounds (in the total extract and in the fractions) were identified by HPLC-MS (3).
- Structural confirmation was developed using pure standards of the most representative compounds

Results and Discussion

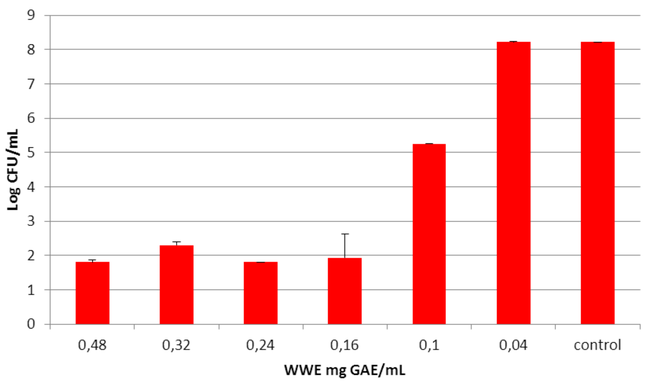
Antimicrobial activity of WW against *Campylobacter*

Table 1. Antibacterial activity of the WW (0.48 mg GAE/mL) against 7 strains of Campylobacter. The results are expressed in log cfu/ml ± standard deviation (SD) (n=3)

			Media log CFU/ml reduction) (n =3)	± SD (log
Campylobacter strains	Control	WW	(0,48 mg GAE/ml)	Log of Inhibition
C. jejuni LP1	8.37±0.21		2.5± 144	5.87
C. jejuni CII I	6.18±0.82		1.59± 3.14	4.59
C. jejuni CN1	8.29 ±0. 23		3.98 ± 0.38	4.31
C. jejuni 118	8.51±0.2		>1,48 [a] ± 0,0	>7.03
C. jejuni 11168	7.88 ±0. 1		6.44± 0.21	1.44
C. jejuni 11351	7.06±0.71		3.35± 0.07	3.71
C. coli LP 2	8.56±0.1		2.44± 1.62	6.12

[a] Calculated log of detection limit (30 cfu/plate)

Figure 1. MIC of WW on viable counts of C. jejuni LP1. Results are expressed as log CFU/ml ± standard deviation (n =2)



Structural analysis of the phenolic composition of the extracts

Figure 2. Chromatogram of the fractions collected by preparative HPLC

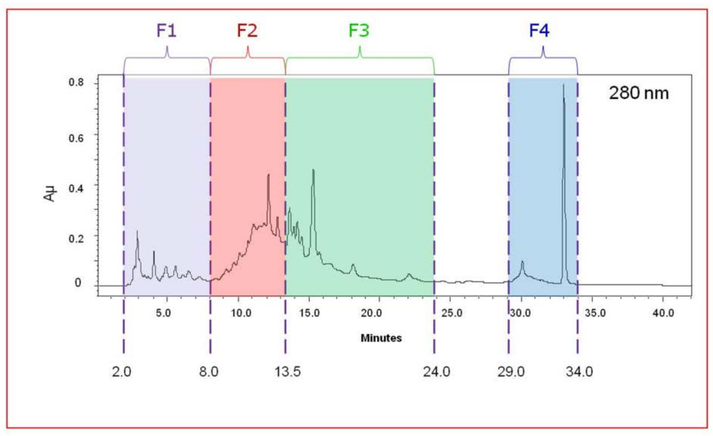


Figure 3. Effect of the fractions on *C. jejuni* . Fraction 2 held a significant antimicrobial activity against the pathogen.

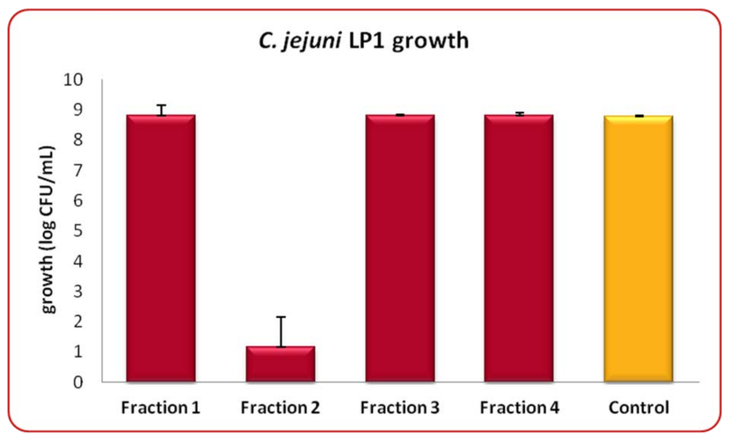


Table 2. Phenolic composition (mg/L) of WW and its collected fractions (mean ± SD) (n=3).

Compounds	Total extract			Fraction 1			Fraction 2			Fraction 3			Fraction 4		
Flavonols	**421.93**	**±**	**4.92**	**0.00**	**±**	**0.00**	**21.64**	**±**	**0.02**	**168.79**	**±**	**0.73**	**0.00**	**±**	**0.00**
Myricetin-3-rutinoside	traces						traces								
Myricetin-3-glucoside	47.61	±	0.41												
Quercetin-3-rutinoside (Rutin)	23.97	±	0.29							18.40	±	0.02			
Quercetin-3-glucoside	94.45	±	0.77							63.37	±	0.22			
Quercetin-3-rhamnoside (Quercitrin)	94.45	±	0.77							7.00	±	0.00			
Quercetin-3-glucuronide	70.29	±	0.65				21.64	±	0.02	38.15	±	0.07			
Kaempferol-3-rutinoside	10.10	±	0.15							5.41	±	0.09			
Kaempferol-3-glucoside	27.97	±	0.52							16.03	±	0.22			
Quercetin	37.87	±	1.14							15.10	±	0.09			
Kampferol	15.24	±	0.21							5.33	±	0,02			
Acids	**837.89**	**±**	**153.23**	**743.70**	**±**	**78.12**	**13.45**	**±**	**0.50**	**0.00**	**±**	**0.00**	**0.00**	**±**	**0.00**
Phloroglucinol	791.50	±	151.51	719.83	±	76.50									
Siringic acid	20.42	±	0.39				12.70	±	0.50						
Galic acid	25.97	±	1.33	23.87	±	1.63	0.75	±	0.00						
Catechins	**578.41**	**±**	**44.30**	**34.16**	**±**	**3.91**	**132.99**	**±**	**3.21**	**0.00**	**±**	**0.00**	**0.00**	**±**	**0.00**
B1	83.32	±	4.30	34.16	±	3.91	17.54	±	0.55						
Catechin (Cat)	88.04	±	6.62				9.78	±	0.54						
B2	110.22	±	9.01				41.08	±	0.21						
Epicatechin (Ec)	156.73	±	12.22				23.55	±	0.79						
Ec-Ec-Cat	96.13	±	10.40				16.23	±	0.54						
Ec- Epicatechin gallate (ECG)	18.09	±	0.21				13.60	±	0.15						
ECG	25.88	±	1.54				11.22	±	0.42						
Anthocyanins	**222.00**	**±**	**1.84**	**0.00**	**±**	**0.00**	**14.49**	**±**	**0.69**	**122.76**	**±**	**0.78**	**0.00**	**±**	**0.00**
Delfhinidin-3-glucoside	23.14	±	0.35				12.68	±	0.58						
Cyanidin-3-glucoside	traces														
Peonidin-3-glucoside	71.32	±	0.62				0.72	±	0.02	36.91	±	0.50			
Malvidin-3-glucoside	125.73	±	0.84				1.10	±	0.09	85.58	±	0.27			
Malvidin-3-acetate	1.81	±	0.04							0.27	±	0.01			
Total Polyphenols	**2060.24**	**±**	**204.29**	**777.86**	**±**	**82.04**	**182.57**	**±**	**4.42**	**291.55**	**±**	**1.51**	**0.00**	**±**	**0.00**

Conclusions

All the strains were sensitive to WW at a concentration of 0.48 mg GAE/ml (table 1). The MIC of WW was carry out on a representative strain. WW was effective against C. jejuni until a concentration of 0.1 mg GAE/L. The fractioning of the sample showed that fraction 2 is the responsible of the behavior observed. This fractions was mainly constituted by phenolic acids and catechins. Using pure standards, we check that siringic acid (phenolic acid), epicatechin and epicatechin-gallate (catechins) were the most active compounds. This information can contribute to design new process to produce phenolic extracts active against *Campylobacter*

References: 1) Ganan, M., Silván, J.M., Carrascosa, A.V., Martinez-Rodriguez, A.J. (2012). Food C*ontrol* 24, 6-14; 2) EFSA. (2010). *EFSA Journal*, 8, 1496.
3) Avila, M., Hidalgo, M., Sánchez-Moreno, C., Pelaez, C., Requena, T., de Pascual-Teresa, S. (2009). *Food Research International*, 42, 1453-1461

Acknowledgements

This research was supported by projects AGL2009-07894 and FUN-C-FOOD 2011 BIOCAMP